Medical Lib.

VOL. IV. No. 1] [FEBRUARY, 1923.

THE BRITISH JOURNAL OF EXPERIMENTAL PATHOLOGY

Editors:

C. L. EVANS
P. FILDES
W. E. GYE
E. H. KETTLE
J. McINTOSH
H. MACLEAN
J. A. MURRAY
J. B. ORR.
W. J. TULLOCH
C. M. WILSON

With the Support of:

F. W. ANDREWES (London)
W. M. BAYLISS (London)
W. BULLOCH (London)
S. L. CUMMINS (Cardiff)
H. H. DALE (London)
A. M. DRENNAN (Otago, N.Z.)
G. DREYER (Oxford)
J. W. H. EYRE (London)
LEONARD FINDLAY (Glasgow)
DUNCAN GRAHAM (Toronto)
R. T. HEWLETT (London)
F. G. HOPKINS (Cambridge)
W. B. LEISHMAN (London)
T. J. MACKIE (Cape Town)
H. OERTEL (Montreal)
LEONARD ROGERS (London)
C. S. SHERRINGTON (Oxford)
A. E. WRIGHT (London)

LONDON: H. K. LEWIS & CO. LTD., 136, GOWER STREET, W.C.1.

EDITORIAL AND PUBLISHING OFFICE: 28, GOWER PLACE, W.C.1.

CANADA: THE MACMILLAN COMPANY OF CANADA, LTD., BOND STREET, TORONTO.

Annual Subscription, January to December, post free, Two Pounds net.
Single Numbers, Seven and Sixpence net.

THE BRITISH JOURNAL OF EXPERIMENTAL PATHOLOGY.

EDITORIAL NOTICE TO CONTRIBUTORS.

Scope of Papers.—This Journal has been founded for the publication of original communications describing the technique and results of experimental researches into the causation, diagnosis and cure of disease in man. Papers upon bacteriological, biochemical, physiological, serological and other subjects are therefore acceptable when bearing directly upon these objects.

Forwarding Papers.—Papers should be addressed to—

THE EDITORS,
British Journal of Experimental Pathology,
28, GOWER PLACE,
LONDON, W.C. 1,

or to any of the Editors personally.

Date of Publication.—The Journal is published bi-monthly, in February, April, June, August, October, and December.

Priority of Publication.—Papers accepted for publication will be printed as far as possible in the order of their receipt by the Editors, but those sent from overseas will receive priority in publication according to the date of posting instead of the date of receipt.

Conditions of Publication.—Contributors should prepare their manuscripts with the greatest attention to detail. The fact of submitting a paper for publication will be taken by the Editors as an admission by the Author of his liability to pay for all unreasonable alterations made by him in proofs, and for mistakes due to lack of clearness in the preparation of the manuscript.

References.—Authors are requested to prepare their references in strict accordance with the method adopted in current issues of this Journal.

Proofs.—To avoid delay, proofs will not be submitted to Authors overseas unless they express a desire to see them, or unless the Editors think it necessary. Overseas Authors are therefore particularly reminded of the importance of preparing their manuscripts in such a way that error need not arise.

Reprints.—25 reprints will be supplied free to authors. Additional copies must be ordered from the Publishers *before the issue is printed,* and will be charged for according to the following scale:

Number of Additional Reprints.

Number of pages.	25			50			75			100			150			200		
	£	s.	d.	£	s.	d.	£	s.	d.	£	s.	d.	£	s.	d.	£	s.	d.
1–4	0	16	6	0	19	6	1	2	6	1	5	6	1	9	6	1	13	6
5–8	1	5	0	1	8	0	1	11	0	1	14	0	1	19	6	2	5	0
9–12	1	16	0	1	19	0	2	2	0	2	5	0	2	12	6	3	0	0
13–16	2	5	0	2	8	0	2	11	0	2	14	0	3	6	0	3	15	0

Authors working in countries where the rates of exchange are unfavourable to them will be charged for reprints according to pre-war rates (25 francs = 20 shillings).

Med.
Faxon

CONTENTS

No. 1.—FEBRUARY.

PAGE

PATTERSON, S. W.—A Complement-Fixation Test in Coccidiosis of the Rabbit . 1

BEDSON, S. PHILLIPS, AND ZILVA, S. S.—The Influence of Vitamin A on the Blood-Platelets of the Rat 5

DODDS, E. C.—Variations in some of the Urinary Constituents and the Alveolar Carbon Dioxide Tension in Relation to Meals 13

GLENNY, A. T., ALLEN, K., AND HOPKINS, BARBARA E.—Testing the Antigenic Value of Diphtheria Toxin-Antitoxin Mixtures 19

AMMON, S. E., AND LIM, R. K. S.—The "Gastrin" Content of the Human Pyloric Mucous Membrane 27

O'BRIEN, R. A., EAGLETON, A. J., OKELL, C. C., AND BAXTER, MISS M.—The Schick Test and Active Immunisation 29

MCINTOSH, JAMES.—The Diagnostic Value of Rabbit Inoculation in *Encephalitis lethargica* 34

No. 2.—APRIL.

CRAMER, W., DREW, A. H., AND MOTTRAM, J. C.—On the Behaviour of Platelets in Vitamin A Deficiency and on the Technique of Counting them . . 37

SOUTHGATE, H. W.—A Suggested Substitute for Canada Balsam as a Mounting Medium 44

DREW, A. H.—Growth and Differentiation in Tissue Cultures 46

LEPPER, ELIZABETH H.—On the Mode of Production of Bacteriophage . . 53

BARNARD, J. E.—The Detection of *Spironema pallidum* 68

ARKWRIGHT, J. A., AND BACOT, A. W. (THE LATE).—Investigation of the Ætiology of Typhus Fever, especially undertaken for the Egyptian Government in the Public Health Laboratories, Cairo 70

No. 2.—APRIL—*continued.*

PAGE

LAZARUS-BARLOW, W. S., AND PARRY, R. H.—The Brain and Immunity Reactions to Jensen's Rat Sarcoma 80

UNDERHILL, S. W. F.—The Relative Concentration Ratios of some Constituents of the Urine. I.—The Distribution between Plasma and Corpuscles, and the Condition in the Blood, of Urea, Creatinine, Inorganic Phosphate and Uric Acid 87

BARRY, D. T.—Vascular Changes affecting Bulbar Nerve Centres . . . 92

No. 3.—JUNE.

HARDING, VICTOR JOHN, AND POTTER, CARR T.—The Excretion of "Acetone" and Nitrogen in Nausea and Vomiting of Pregnancy 105

UNDERHILL, S. W. F.—The Relative Concentration Ratios of Some Constituents of the Urine. II.—The Relative Concentration Ratios of Urea, Creatinine, Inorganic Phosphate and Uric Acid 117

FYFE, G. MATTHEW.—A Method of Estimation of Diastase in Blood . . 127

MELLANBY, JOHN, AND ANWYL-DAVIES, THOMAS.—The Precipitation of Colloidal Gold by Cerebrospinal Fluid; the Diagnosis of Neuro-Syphilis . . 132

DREYER, GEORGES.—Some New Principles in Bacterial Immunity; their Experimental Foundation, and their Application to the Treatment of Refractory Infections 146

No. 4.—AUGUST.

PATON, D. NOËL, AND WATSON, ALEXANDER.—The Ætiology of Rickets: An Experimental Investigation.—Part II 177

AHLGREN, GUNNAR.—Experiments on the Respiration of Malignant Tissues . 196

LEPPER, ELIZABETH H.—The Rate and Progress of Bacteriophage Action . 204

CRUICKSHANK, E. W. H.—Studies in Experimental Tetany: I. Distribution of Calcium. II. Colloidal and Ionic Calcium 213

MCINTOSH, JAMES, AND KINGSBURY, A. NEAVE.—The Expression of the Results of the Wassermann Reaction in Units 224

HINE, T. G. M.—Auto-dissociation of Agglutinin-Antigen Complex . . . 231

MARTLAND, E. MARJORIE.—The Complement-Fixation Test in the Diagnosis of Gonococcal Infection in Women 235

No. 5.—OCTOBER.

PAGE

COWAN, MARY L.—Variation Phenomena in Streptococci: Further Studies on Virulence and Immunity 241

LAZARUS-BARLOW, W. S., AND PARRY, R. H.—The Spleen and Immunity Reactions to Jensen's Rat Sarcoma 247

KELLAWAY, C. H., AND COWELL, S. J.—Spontaneous Desensitisation . . 255

MARRACK, JOHN, AND BOONE, WILLIAM BROOKE.—The Acid-base Balance in the Plasma in the Later Stages of Pregnancy 261

FILDES, PAUL.—The Classification of Hæmoglobinophilic Bacteria, Based upon their Relation to Blood-Pigment and to the "Vitamine" Factor . . 265

CRAMER, W., AND DREW, A. H.—The Effect of Light on the Organism. . . 271

GLENNY, A. T., AND HOPKINS, BARBARA E.—Diphtheria Toxoid as an Immunising Agent 283

No. 6.—DECEMBER.

EAGLETON, A. J., AND BAXTER, E. M.—The Standardisation of Tuberculin . . 289

BEDSON, S. PHILLIPS, AND ZILVA, S. S.—The Platelet-Count in Rats Suffering from Vitamin A Deficiency 305

BABKIN, B. P.—The Influence of Insulin on the Formation of Glycogen . . 310

STONE, G. KENNETH.—Complement-Fixation in Streptococcal Infections . . 318

ATKIN, E. E.—Some Cultural Characteristics Exhibited by Serological Types of Meningococci 325

MURRAY, IAN.—The Thyroid Gland in Experimental Rickets 335

CRUICKSHANK, E. W. H.—Variations in the Distribution of CO_2, Chlorine and Calcium in the Cells and Plasma of the Blood in Tetany 340

INDEX 347

THE BRITISH JOURNAL

OF

EXPERIMENTAL PATHOLOGY

A COMPLEMENT-FIXATION TEST IN COCCIDIOSIS OF THE RABBIT.

S. W. PATTERSON, M.D., D.Sc.

Director, Walter and Eliza Hall Research Institute, Melbourne.

Forwarded for publication October 16th, 1922.

RECENTLY an epidemic of coccidiosis (*Eimeria stiedæ*, Lindemann, 1865) occurred amongst the rabbits bred for experimental purposes in the Institute, and as a result about forty young rabbits succumbed. Many others were affected but are recovering. As a means of sorting out the affected from those non-affected, the complement-fixation test suggested itself. On searching the literature, I find that Kuczynski (1921) discovered that the serum of rabbits spontaneously or intentionally infected with coccidia gave a positive Wassermann reaction. He believes that the fact, recorded by Friedemann and by Wendtlandt, that rabbit serum often gives a positive reaction, is to be explained by the frequency of coccidiosis amongst these animals.

TECHNIQUE.

Antigens.—As coccidiosis affects mainly the liver and the mucous membrane of the upper part of the small intestine, the antigens used were prepared by extracting the infected livers with absolute alcohol, with carbolised normal saline solution, and with normal saline (0·85 per cent.) solution.

(1) Alcoholic extract: 5 grm. heavily infected liver were ground in a mortar with an equal quantity of kieselgühr, and 40 c.c. of absolute alcohol added. The mixture was put in a stoppered flask and incubated at 37° C. for two days, being shaken several times a day. It was filtered and the filtrate was kept on ice till required. A clear yellow fluid was obtained.

(2) Carbol-saline extract: This was prepared as in No. 1 antigen, using, however, normal saline (to which 0·5 per cent. of carbolic acid had been added) as a solvent instead of alcohol. A fairly clear yellow filtrate was obtained.

(3) Normal saline extract: 5 grm. infected liver were rubbed up with kieselgühr and 40 c.c. of normal (0·85 per cent.) saline in a mortar, and the mixture was placed on ice in a closed flask and shaken thoroughly several times a day. It was filtered before use, but a very turbid filtrate resulted.

Hæmolytic systems.—Hæmolysins prepared from rabbits against sheep and ox-corpuscles respectively were used with the corresponding cells; the cells received maximum sensitisation with 4 M.H.D. of hæmolysin.

Complement.—Fresh serum from healthy male guinea-pigs was used as complement. This was titrated as a preliminary with the hæmolytic system, and used in a dilution so that unit volume (2 drops) contained 3 M.H.D.

The dropping method was used throughout, 2 drops (unit volume) being 0·2 c.c. with the pipettes used.

Preliminary tests were carried out to determine the inhibition of complement by the antigen alone. Two drops of antigen in diluted series were put up with 4 drops of saline solution and 2 drops of diluted complement (3 M.H.D.). After incubation for one hour at 37° C., 2 drops of the sensitised cells were added, and the degree of hæmolysis read at the end of another hour in the incubator at 37° C. Table I shows the results obtained in one experiment.

TABLE I.

Hæmolytic system.	Antigen.	Dilution. 1	1/2	1/3	1/6	1/9	1/12	1/24	1/36
Sheep—anti-sheep	alcoholic	+++	+++	+	−	−	−	−	−
	carbol-saline	−	−	−	−	−	−	−	−
	normal saline	+	−	−	−	−	−	−	−
Ox—anti-ox	alcoholic	+++	+++	−	−	−	−	−	−
	carbol-saline	−	−	−	−	−	−	−	−
	normal saline	+	−	−	−	−	−	−	−

− = complete hæmolysis, + = almost complete hæmolysis, ++ = half cells hæmolysed, +++ = no hæmolysis.

For the test then the alcoholic extract was used in a dilution of 1 to 9 parts of normal saline solution, and the carbol-saline and normal saline extracts in dilutions of 1 to 2 parts of normal saline. The test was set up with four tubes for each serum, which was diluted 1 in 5 with saline and inactivated for 20 minutes in a water-bath at 56° C. The first three tubes contained serum, antigen, and 3, 4½, and 6 M.H.D. of complement respectively, while the fourth tube, the control, contained serum and 3 M.H.D. of complement. Each tube was made up to 0·8 c.cm. with saline. Controls of complement and complement with antigen and with double-strength antigen were set up in each set. The sets were put in the incubator at 37° C. for one hour, then 0·2 c.c. of sensitised red cells added, and the incubation continued for one hour longer.

TABLE II.

Extract	Alcoholic 1/10.						Carbol sal. 1/3.						Normal sal. 1/3.					
Hæmolytic system	Sheep.			Ox.			Sheep.			Ox.			Sheep.			Ox.		
Complement M.H.D.	3.	4½.	6.	3.	4½.	6.	3.	4½.	6.	3.	4½.	6.	3.	4½.	6.	3.	4½.	6.
Rabbit, infected	−	−	−	−	−	−	+	+	−	+	+	−	+	+	−	−	+	−
" "	+	−	−	−	−	−	+	+	−	+	+	−	+	+	−	−	+	+
" "	−	−	−	−	−	−	+	+	−	+	+	−	+	+	−	−	+	−
" "	+	−	−	−	−	−	+	+	−	+	+	−	+	+	−	−	+	−
" "	+	−	−	−	−	−	+	+	−	+	+	−	+	+	−	−	+	−
" "	+	−	−	−	−	−	+	+	−	+	+	−	+	+	−	−	+	−
" recovering	−	−	−	−	−	−	+	+	−	+	+	−	+	+	−	−	+	−
" normal	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
" "	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
" old scarred liver	+	−	−	−	−	−	+	+	−	+	+	−	+	+	−	−	+	−
Guinea-pig	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Human, positive Wassermann reaction	+	+	−	−	−	−	+	−	−	−	−	−	+	+	−	−	−	−
Human, positive hydatid reaction	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Control; complement alone,																		
do. + antigen, do. + double strength antigen	−	−	−	−	−	−	−	−	+	−	−	−	−	−	++	−	−	−

The sera of at least two rabbits known to be healthy were put up at each test.

A typical protocol obtained is shown in Table II (p. 3).

RESULTS.

Sera from three rabbits proved to be normal and from twenty-nine other rabbits known or suspected to be infected were examined, and the results of the series of tests are summarised in Table III.

TABLE III.

	Antigen	Alcoholic extract.		Carbol-saline.		Normal saline.	
	System	Sheep.	Ox.	Sheep.	Ox.	Sheep.	Ox.
Normal	No. tested	3	3	3	3	3	3
	Strong reaction	0	0	0	0	0	0
	Reaction	0	0	0	0	0	1
	No reaction.	3	3	3	3	3	2
Infected	No. tested	17	17	29	29	29	29
	Strong reaction	0	0	9	12	17	17
	Reaction	2	2	5	8	9	11
	No reaction.	15	15	15	9	3	1

The alcoholic extract, which fixed complement in the presence of human serum giving the Wassermann reaction, proved a poor antigen even in dilutions of 1 in 6, and in stronger amounts tended to fix complement itself. The carbol-saline extract of the infected livers had good antigenic properties, and showed no anticomplemetary tendency. The normal saline extract acted as a good antigen, but tended to become anticomplementary on keeping.

Note.—I have to acknowledge the great assistance of Miss F. E. Williams in carrying out the technical part of the work.

REFERENCE.

KUCZYNSKI, M. H.—(1921) *Berlin. klin. Wchnschr.*, **58**, 125.

THE INFLUENCE OF VITAMIN A ON THE BLOOD-PLATELETS OF THE RAT.

S. PHILLIPS BEDSON, M.D., M.Sc.DUNELM,

AND

S. S. ZILVA, D.Sc.

From the Bacteriological and Biochemical Departments, Lister Institute, London.

Received for publication January 3rd, 1923.

IN a recent communication Cramer, Drew and Mottram (1922) describe a series of experiments in which a specific lesion, not previously observed, is manifested in rats kept on a diet free from vitamin A. This lesion is characterised by a reduction in the number of blood-platelets which was found to vary with the intensity of the dietetic deficiency. One need hardly enlarge on the physiological significance of such a phenomenon if it could be confirmed. Our interest in this work was particularly stimulated by the possibility of utilising this observation in the quantitative estimation of vitamin A. It has been already pointed out by Zilva and Miura (1921) that in the case of a vitamin A deficiency there is no clear-cut symptom as in the case of scurvy and polyneuritis, which can be utilised as an "end-point" in the quantitative estimation of the vitamin. Resumption of growth by rats fed on a vitamin A deficient diet under specified conditions mainly devised to eliminate vigorously growing animals in the initial stages of the deficiency was, therefore, suggested by them as a standard. Good results have been obtained by this method. It is recognised, however, that the employment as a standard of a more decisive and specific lesion, such as a progressive thrombopœnia, would greatly enhance the quantitative method described by Zilva and Miura. According to Cramer, Drew and Mottram, a diminution in the number of platelets can be recorded in vitamin A deficient rats independent of their growth propensities, and this change becomes markedly manifest before the infective conditions make their appearance. The average of the platelet counts obtained by these workers in normal rats is 851,000 per c.mm., whilst the average of their counts in vitamin A deficient rats is 378,000, the lowest platelet recorded by them in this latter category of rats being 154,000 per c.mm.

Our preliminary observations were made with the object of corroborating these findings of Cramer, Drew and Mottram. Platelet counts were made on vitamin A deficient rats and compared with counts in normal rats of the same age. No marked divergence between the platelet counts in these two

categories of rats was observed. This failure urged us to repeat Cramer, Drew and Mottram's work in detail. These authors find that the platelet count falls not only in rats which show little or no initial growth immediately after being placed on the deficient diet, which animals they designate for the sake of convenience as "acute," but also in rats which show a marked tendency to grow for some time before the deficiency is demonstrated by their weight curve and which are described as "chronic." On administering the fat-soluble factor the number of platelets increased and eventually reached the normal count. We therefore investigated the three categories of animals.

The deficient diet of our animals consisted of the following ingredients:

Inactivated casein	20 parts.
Starch	50 „
Salt mixture	5 „
Marmite	5 „
Decitrated lemon-juice . . .	5 „
Hardened cotton-seed oil . . .	15 „

The casein was inactivated by being heated for 48 hours in shallow trays and frequently turned over at a temperature of 120° to 130° F. The salt mixture employed was of the same composition as that used by McCollum and his colleagues. This diet was found from several years' experience to be very deficient in vitamin A, and is used extensively for the quantitative estimation of this vitamin.

TECHNIQUE OF THE PLATELET COUNT.

The method employed during this investigation for the enumeration of the platelets is the same in principle as the one described in a previous communication (Bedson, 1921). The ratio of red cells to platelets is first obtained in a preparation of citrated blood mounted between slide and cover-glass, and the absolute red count is then made by means of the Thoma-Zeiss pipette and counting chamber. By means of these data the absolute platelet count is calculated. In view of the fact, however, that some parts of the technique had to be modified to render it applicable to the rat, it would be perhaps advisable to describe the method in detail.

Method for obtaining the blood for the red-cell-platelet ratio.—The blood for this estimation was obtained by one of two methods:

(1) By cardiac puncture.

(2) By cutting the tail whilst immersed in an anti-coagulant.

In carrying out the first procedure (cardiac puncture) the rat is anæsthetised with ether and the chest-wall shaved. A small all-glass syringe fitted with a comparatively wide-bore needle is used. This is thoroughly washed out with normal saline containing 2 per cent. of sodium citrate and then 0·5 c.c. of the same citrate-saline solution is drawn up into the syringe and forced to the tip of the needle. The point at which to insert the needle is readily determined by palpation. The needle is plunged into the heart, about 0·05 c.c. of blood drawn up and the needle withdrawn. The blood is then rapidly mixed with the citrate by rotating the syringe, the needle removed and the citrated blood

expelled into a paraffined watch-glass. In order to obtain a satisfactory preparation by this method the needle must enter the heart at the first attempt, and as soon as the blood has entered the syringe it must be thoroughly mixed with the diluent with as little delay as possible. Should the first attempt at cardiac puncture not be successful, then the needle must be withdrawn and thoroughly washed out with the citrate solution.

The second method is the one described by Cramer, Drew and Mottram. It consists of cutting the tail whilst immersed in the diluent (2 per cent. citrate in saline). As soon as the blood is flowing freely, the tail is transferred to a second quantity of citrate saline and allowed to remain there until the requisite amount of blood has been obtained. These operations are carried out with the rat deeply etherised. This is essential if a free flow of blood is to be obtained, as Cramer, Drew and Mottram have pointed out.

In our hands the first of these two methods, viz. cardiac puncture, has proved the most satisfactory. The risk of any action on the platelet by tissue juices is reduced to a minimum, and there is not the difficulty of getting the animal to bleed, which is sometimes the case with the "tail" method. However, cardiac puncture has its limitations. It is very difficult to perform on a young rat of only 50 grm. weight, and it is not applicable to those cases where a series of counts is to be made on an individual rat, owing to the risk of killing the animal. This being so, we have made use of this method only where isolated platelet counts were being made, and when it was desired to make a series of observations on individual rats the blood has been obtained from the tail.

The red-cell-platelet ratio estimation.—The blood, obtained by one or other of the methods just described, is thoroughly mixed, and a suitable quantity transferred to a slide by means of a paraffined pipette or platinum loop, covered immediately with a cover-slip and ringed with vaseline. The quantity of blood taken should be such as to spread out evenly between the slide and cover-slip without causing the latter to float. The number of platelets and red cells in a series of fields of this preparation is then counted. The size of field selected should not be too large, otherwise the number of red cells to be counted becomes too great. Needless to say, also, the preparations should show no clumping of platelets, and the dilution of the blood should be such as to give well-isolated elements. However carefully the preparation be made it will be found that the distribution of the platelets is not absolutely regular. In view of this fact *a series of fields is counted right across the preparation from one side to the other.* If sufficient platelets have not been counted in traversing the preparation once, then it must be moved either up or down and traversed again in the opposite direction. *At least 100 platelets must be counted.* The counting can be done either with a 4 mm. objective and fairly high ocular or with the oil immersion. The illumination can be either by means of a dark-ground condenser, or by an ordinary condenser with the iris diaphragm closed sufficiently as to give clear definition. The actual counts which form the subject of this communication were made either with objective 6 and ocular 4 (Koristka), or with objective $\frac{1}{15}$″ semi-apochromat. and compensating ocular 8 (Koristka). An Abbe condenser (N.A. 1·3) was used in both instances. In many cases the same preparation has been counted both with the dry lens and

the oil immersion and similar counts obtained. By counting the preparation right across from side to side and by always moving the preparation on with the eye removed from the microscope—thus avoiding any selection of field—the personal factor has been reduced to a minimum.

EXPERIMENTAL FINDINGS.

Our first observations, as has been pointed out, consisted of isolated platelet counts on normal and vitamin A deficient rats. These rats were all of approximately the same age—a point of importance when one remembers how rapidly the blood picture changes in the first few months in the life of these animals. The figures obtained are tabulated below (Tables I and II). The blood for the red cell-platelet ratio determination was obtained in every instance from the heart. The counting was done with objective 6 and ocular 4 (Koristka), and in certain instances the counts so obtained were checked by recounting the preparation with objective $\frac{1}{15}$ inch and compensating ocular 8 (Koristka). As stated already, no appreciable difference was found between the counts made with these two different magnifications.

TABLE I.—*Platelet Counts obtained in 7 Normal Rats.*

Red cell.	R.P. ratio.	Platelets.
6,896,000	6·3	1,094,000.
7,776,000	6·8	1,143,000.
7,840,000	7·0	1,120,000.
8,040,000	5·7	1,585,000.
8,448,000	7·2	1,173,000.
8,080,000	7·5	1,077,000.
7,520,000	5·9	1,270,000.
Average of above counts.		
7,800,000		1,208,857.

It will be observed that the figures obtained for the platelets in these seven rats vary between extremes of 1,585,000 and 1,077,000 per c.mm. The average count, 1,208,857 is considerably higher than what is found in the guinea-pig and rabbit.

TABLE II.—*Platelet Counts in 9 Vitamin A Deficient Rats.*

Red cells.	R.P. ratio.	Platelets.	Remarks.
7,800,000	5·4	1,440,000	Ceased growing. No eye change.
8,360,000	6·8	1,229,000	,, ,,
7,736,000	6·5	1,190,000	Well-developed keratomalacia.
7,120,000	8·7	818,000	Moderate degree of ,,
7,568,000	6·5	1,164,000	Well-developed ,,
8,336,000	9·28	898,000	,, ,,
7,760,000	8·6	902,000	,, ,,
7,568,000	7·7	982,000	,, ,,
8,272,000	7·8	1,060,000	,, ,,

Average of above counts.

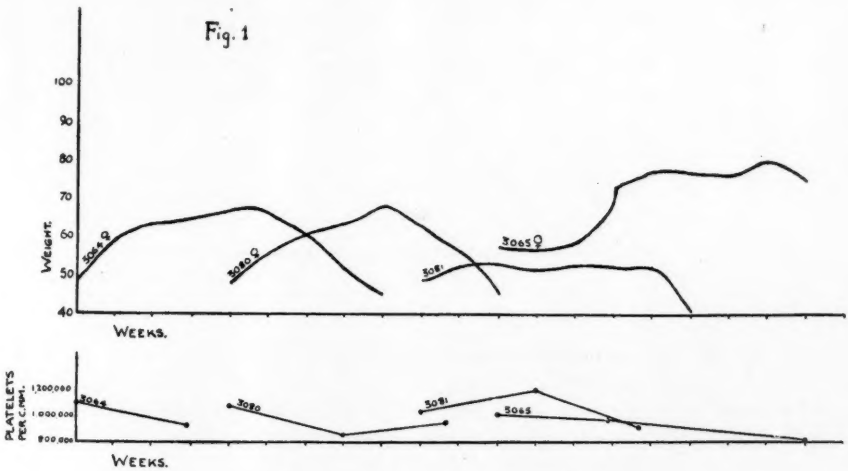
7,946,666 1,075,888

Average of counts in those rats with keratomalacia.

7,908,555 1,002,000

The platelet counts in these nine rats are, generally speaking, slightly lower than those obtained in normal rats, and this is certainly more evident in some of those animals which had developed definite eye change. The difference between the average of these nine counts and the seven normal counts is only 122,969, or if we exclude the two deficient rats which had not

Fig. 1



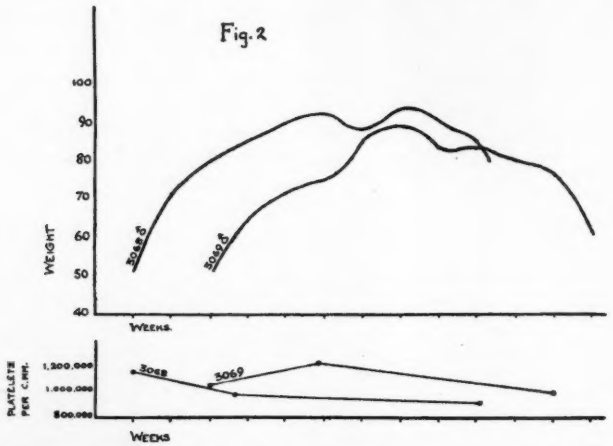
developed keratomalacia it is 206,857. Although the difference is probably more than could be accounted for by experimental error, it by no means represents the very considerable fall in the platelet count experienced by Cramer, Drew and Mottram. If the withholding of vitamin A from rats had as its specific result a considerable diminution in the number of platelets, then one would have expected the above counts, representing as they do the two extremes of the picture, to have revealed a considerable difference between normal and deficient rats. It was recognised, however, that a more satisfactory procedure would be to make a series of platelet counts on rats both before and during the course of a vitamin A deficiency. This was carried out on six rats, and the findings both as regards the weight and the platelets have been charted as curves in Fig. 1 and 2. In making these counts the blood has been obtained from the tail, and the counting carried out with objective Koristka $\frac{1}{15}$ inch semi-apochromat. and compensating ocular 8.

Fig. 1 represents the weight curve of animals which can be classified as "acute," whilst in Fig. 2 the growth of rats belonging to the "chronic"

category is graphically portrayed. It is to be pointed out that the "chronic" animals did not attain such a high weight as their prototypes in Cramer, Drew and Mottram's experiments. This is due to the fact that they were started on the deficient diet at a lower weight. Possibly they may also not have had the same reserve of the vitamin. Their rate of growth, however, was distinctly more intense than that of the "acute" group.

An examination of the data recorded in Figs. 1 and 2 reveals the fact that here again, though vitamin A deficiency is accompanied by a rather low platelet count, the fall recorded is very small.

Fig. 2



In order to bring out this point more clearly the actual platelet counts, together with the percentage fall in each case, are given below (Table III).

TABLE III.

Rat.	Platelet count before commencing experiments.	Final platelet count.	Difference between two counts.	Percentage change.	Time on −A diet between two counts.
3064	1,126,000	924,000	−202,000	17·9	3 weeks.
3065	1,009,000	807,000	−202,000	20·0	8 ,,
3068	1,143,000	899,000	−244,000	21·3	9 ,,
3069	1,054,000	996,000	−58,000	5·5	9 ,,
3080	1,090,000	953,000	−37,000	3·3	5·75 ,,
3081	1,031,000	847,000	−184,000	17·8	5·75 ,,

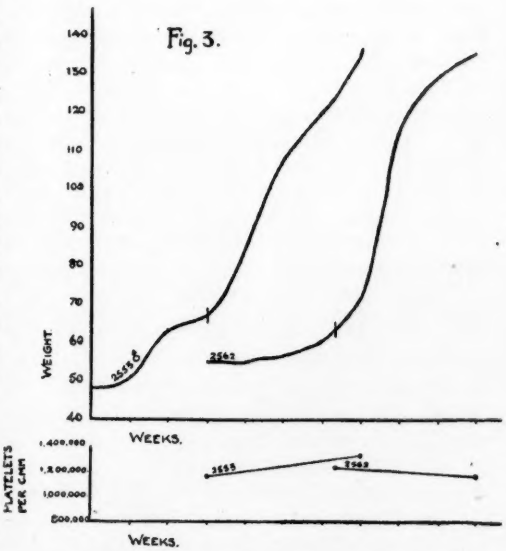
It will be seen that the greatest fall recorded, that in rat No. 3068, is only one of 244,000, or a 21·3 per cent. change, and that in three other rats, Nos. 3064, 3065 and 3081, a fall of somewhat similar degree has taken place. In the remaining two rats there is little change, though in these, again, the change is on the negative side. In order to adjudge small changes in the platelet count, such as we are dealing with, some idea of the experimental error in the

technique employed is necessary. Three counts were, therefore, made on a normal rat at intervals of an hour, using the same technique as had been employed for the above estimations. The following figures were obtained (Table IV):

TABLE IV.

Count.	Platelets per c.mm.
1 . .	1,195,000
2 . .	1,128,000
3 . .	1,321,000

The mean of these three counts is 1,214,000, and the average deviation from this mean 70,666, or 5·8 per cent. It is, of course, recognised that the number of observations is really too small to draw any satisfactory conclusion therefrom. The calculated error probably represents too low an estimate, especially when one remembers that this platelet count is subject to the error of the red cell count as well. The error is most probably in the neighbourhood of 10 per cent., not less, and if we accept this estimate then four out of the six counts recorded in Table III show a fall which lies beyond the limits of experimental error, while the remaining two are certainly within these limits. However, all the changes recorded in the six deficient rats are in the same direction—a diminution—and the probability is that this represents an actual change.



Finally, two vitamin A deficient rats were counted and then cod-liver oil was added to their diet. At the end of four weeks, when these two rats had gained very considerably in weight, their platelets were again counted. The findings, recorded graphically in Fig. 3, speak for themselves.

DISCUSSION.

A consideration of our findings reveals the fact that young growing rats when deprived of vitamin A show a slightly lower platelet count than do normal rats. In those of our experiments where consecutive counts were made, the general tendency of the platelet count is in the direction of a diminution, but even here, accepting the experimental error as 10 per cent., only four out of the six rats show a fall beyond this limit. The greatest

diminution recorded by us was 244,000—a fall of 21·3 per cent. This is considerably less than the diminution in the number of platelets observed by Cramer, Drew and Mottram, and in view of our findings we feel unable to associate ourselves with their opinion that a "thrombopœnia" is a specific lesion of a vitamin A deficiency. We are not at all convinced that this minimal change in the platelet count could not be produced by other dietetic deficiencies, and the few observations on this point brought forward by Cramer, Drew and Mottram do not seem to us of very great value. In the first place they consist of isolated observations only, and secondly, no mention is made of the age of the rats which were examined after being placed on diets deficient in vitamin B. This question of age seems to us of great importance. As is well known, the blood picture in the rat undergoes very considerable changes in the first three months of life. These changes concern more particularly the number of formed elements per unit volume—that is to say, that in the young growing rat the rate of production of formed elements outstrips the production of plasma, with a consequent increasing concentration of formed elements. It is therefore possible that the low platelet counts observed in young growing rats deprived of vitamin A are merely due to the fact that platelet production is slowed rather more than the production of the other elements of the blood, and that a similar change would not be observed in mature rats fed on the same vitamin A free diet. In order to prove this point it would be necessary to work out carefully the platelet content in the blood of rats at frequent intervals during the first three months of life, and also to examine the blood of mature rats deprived of vitamin A. This has not been done. We can offer no explanation of the discrepancy between our findings and those of Cramer, Drew and Mottram.

CONCLUSIONS.

(1) Young growing rats fed on a vitamin A deficient diet show a lower platelet count than do normal rats of the same age.

(2) The greatest diminution in the platelet count observed by us in rats deprived of vitamin A was 244,000, representing a 21·3 per cent. reduction.

(3) We do not consider that this minimal change in the platelet count of vitamin A deficient rats can be regarded in the light of a specific lesion.

The expenses of this research were defrayed from a grant made by the Medical Research Council, to whom our thanks are due.

REFERENCES.

Bedson, S. P.—(1921) "Blood-Platelet Anti-serum, its Specificity and *Rôle* in the Experimental Production of Purpura," *J. Path. & Bacteriol.*, **25**, 94.

Cramer, W., Drew, A. H., and Mottram.—(1922) "On Blood-Platelets; their Behaviour in 'Vitamin A' Deficiency and after 'Radiation,' and their Relation to Bacterial Infections," *Proc. R. Soc.*, B, **93**, 449.

Zilva, S. S., and Miura, M.—(1921) "The Quantitative Estimation of the Fat-Soluble Factor," *Biochem. J.*, **15**, 654.

VARIATIONS IN SOME OF THE URINARY CONSTITUENTS AND THE ALVEOLAR CARBON DIOXIDE TENSION IN RELATION TO MEALS.*

E. C. DODDS.

From the Bland-Sutton Institute of Pathology, Middlesex Hospital, London.

Received for publication January 4th, 1923.

THIS communication, which forms part of a series of investigations into the means adopted by the body to keep the reaction of the blood constant during meals, deals with the urinary factors. As much work has already been done on this subject, it will be as well to summarise the results obtained by previous workers.

PREVIOUS OBSERVATIONS ON THE REACTION OF URINE.

Variations in the urinary acidity have been known for a very long time, but Bence Jones was certainly the first to give any definite information on the subject. His original articles could not be consulted, yet it appears that he described an alkaline tide following meals, ascribing this to the secretion of HCl, thus leaving the blood with a tendency to alkalosis. The excretion of alkali into the urine would tend to correct this. Later Quincke (1884) stated that the changes were not constant, and that the most marked variations were diurnal in origin, being completely dissociated from the ingestion of food. Since these times a great deal of work has been done, some observers (Campbell, 1920) stating that an alkaline tide following meals occurs in practically all cases, whilst on the other hand, it has been held (Leathes, 1919) that this tide either does not exist or appears very rarely. In the opinion of the writer previous workers have run very great risks in the selection of their subjects, since in none of the literature is there any mention of how these investigators knew that their subjects secreted HCl. Bennett and Ryle (1921), in their 100 normal gastric analyses, encountered 4 per cent. of complete achlorhydrics, and the writer, in the course of a routine examination of patients and students in conjunction with Dr. Bennett, has been surprised with the large number of men, to all intents and purposes normal, who showed this condition.

Achlorhydria is so common that it must be excluded by a test-meal before a subject can be accepted for any form of gastric research. Everyone examined for urinary changes had undergone the continuous test-meal, and was thereby proved to have a normal secretion of acid.

* A Report to the Medical Research Council.

METHODS.

The effect of the breakfast and lunch was investigated. In the case of the former, the subject was instructed to pass all his urine on retiring for the night, and to note the time. On rising in the morning the bladder was emptied and the time was again noted. After shaving, etc., he passed the urine secreted since the last specimen, and both of these were put into separate bottles, labelled, and their time intervals very carefully noted. Breakfast was then taken, the time again being carefully noted. He then came up to the laboratory with his two specimens, and passed urine into separate vessels at stated intervals of time. Some of these observations were prolonged for ten hours, others for shorter periods. In the case of the mid-day meal, specimens were collected before and after the meal as described above. Wherever possible, simultaneous alveolar CO_2 tensions were determined; but unfortunately these could not be obtained in the case of breakfast observations. The meals were made as standard as possible, consisting of, for breakfast, half a pint of tea, bacon and eggs, bread and butter; for lunch, meat, potatoes, bread, cheese, and half a pint of water were taken. During the observations the subjects were at rest in the laboratory, reading.

ANALYSIS OF SPECIMENS.

The volume of each specimen was recorded, and the "titrable acidity" and "ammonia" values were determined in the following way:

To 5 c.c. of urine 20 c.c. of boiled distilled water was added, followed by 0·75 c.c. of cresol red as an indicator. Neutral potassium oxalate (10 gm.) was added in order to precipitate those salts which tend to obscure the end-point. A standard solution of pH 7·4 was prepared by means of Sörensen's buffer phosphate solutions, and 0·75 c.c. of the cresol red solution added. The resulting solution was of a brownish colour, and was placed in a comparator. The urine was titrated to the colour standard by the addition of N/100 acid or alkali, according to whether it was acid or alkaline.

All comparisons were made in the comparator.

The titrable acidity having been determined, the ammonia content was arrived at by Malfatti's (1908) formalin method. To the solution obtained above 10 c.c. of 20 per cent. formalin was added, the formalin having been neutralised in the manner described for the estimation of titrable acidity. Owing to a peculiar obscuring action of the formalin on the indicator, Folin's (1919) air current method had to be used in a number of cases. Analyses were carried out on the day of the experiment whenever possible, and, in the case of delay, the urine was preserved by the addition of 1 c.c. of chloroform.

CALCULATION OF THE RESULTS.

It is at once obvious that the figures obtained by expressing the acidity and ammonia content as percentage c.c. N/100 alkali are not comparable in the various specimens. No account is taken of the varying intervals of time, which, owing to the nature of the experiments, are not always regular, nor of the amount of urine passed in that time, which naturally varies. Perhaps the fairest way to express the results is by excretion of acid or ammonia per

hour, which embraces both the above factors. This value is arrived at as follows:

Let X c.c. be the quantity passed in Y minutes.

Let a be the number of c.c. of N/100 acid required to neutralise 100 c.c. of urine, *i. e.* the percentage acidity.

Then the amount of urine passed per hour will be $\frac{60\ X}{Y}$ c.c.

Since the percentage acidity is a c.c. N/100, the acid excretion per hour will be $\frac{a}{100} \times \frac{60\ X}{Y}$ c.c.

The ammonia excretion per hour is arrived at in the same manner.

The A and N value, *i. e.* titrable acidity per hour *plus* ammonia as c.c. $\frac{N}{100}$ alkali per hour, is regarded by Henderson as a useful index of the "acid" or "alkaline" tendencies of the body, and these, together with the CO_2 tension readings, will be found in the following tables:

As the results seem rather irregular, it was thought that the actual pH of the urine might give some valuable information. This was determined in some of the later cases by means of indicators and is appended to the respective tables.

RESULTS OF PRESENT OBSERVATIONS*: THE URINE IN RELATION TO MEALS.

Stated briefly, the present observations show that an alkaline tide follows the first meal of the day. After this period of decreased acid excretion, which lasts usually about one to two hours, an acid tide appears, lasting for two to three hours. Now as lunch is usually taken about four hours after breakfast, any urinary changes which would follow this meal are obscured by the acid tide of breakfast; hence the alkaline tide following this meal is either absent, or shows itself as a very slight drop in the A and N value.

(1) *The breakfast effect.*—Case 1, Table I. The alveolar carbon dioxide tension follows its usual course, the rise being 8 mm.—rather greater than usual. After the meal the urine becomes immediately alkaline, and there is a steady drop for 2½ hours. The hourly excretion of acid and NH_3 show a very steady drop, and then a rise, reaching a maximum 3½ hours after the meal. Six normal men were examined in this manner, and the results were the same in each case. One only is published (Case 1). It will be noted that the rise in the A + N value occurs at the time when lunch is usually taken, consequently any effect that this meal would have on the urine would be obscured by the breakfast effect. To investigate this point a series of all-day observations were made. Six experiments of this type were made, and two are given in the table (Cases 3 and 4). The breakfast effect is as already described, but the lunch produces either no effect at all, or at the most a very temporary drop in the A + N value. Hence, if the lunch effect be investigated, very misleading results are obtained, as can be seen from Case 5, one of a series of seven.

* Owing to the expense of publishing large tables only typical results are reproduced for each group of experiments. It is hoped to publish the whole series of tables in a later and fuller report. Part of the cost of publishing this paper is being borne by the Medical Research Council, to whom the writer wishes to express his thanks.

TABLE I.

	Minutes.	Alv CO_2 mm. Hg.	Volume.		Acidity (Ph 7·4) c.c. N/100 alkali.			NH_3 c.c. N/100.			A+N per hour.	pH.
			c.c. total.	c.c. per hour.	Per cent.	In whole amount.	Per hour.	Per cent.	In whole amount.	Per hour.		
CASE 1.												
7.0–9.0	120	38·76	50	25	290	145	72·5	395	197·5	98·75	171·3	5
Meal 9–9.15												
9.0–9.35	35	41·04	35	60	−28	−9·8	−16·8	123	43·05	91	74·2	6·8
9.35–10.5	30	41·8	50	100	−19	−9·5	−19	83	41·5	83	64	8·2
10.5–10.35	30	44·08	90	180	−20	−18	−36	27	24·3	48·6	12·6	7·5
10.35–11.5	30	46·36	39	78	−65	−25·4	−50·8	45	17·5	35	−15·8	9·5
11.5–11.35	30	41·80	62	124	−18	−12·2	−24·4	66	41	82	59·6	7·9
11.35–12.5	30	40·28	43	86	−0·2	−0·1	−0·2	129	55·5	111	109·8	7·5
12.5–12.30	25	38	36	86	21	7·5	18	189	68	163	181	6·8
12.30–1.0	30	32·68	20	40	116	23·2	46·4	314	62·8	125·6	172	5
1.0–1.30	30	39·1	45	90	194	87·1	174·2	305	137·3	274·6	448·8	4·5
1.30–2.0	30	..	11	22	198	21·8	43·6	437	48	96	139·6	4
CASE 2.												
7.40–8.10	30	..	20	40	376	75·2	150·4	450	900	180	330	5
8.10–8.40	30	..	44	88	160	70·5	141	265	117	234	375	6
8.40–9.20	40	..	24	36	450	108	150	729	175	283	433	5
9.20–10.10	50	..	26	31·2	477	124	149	963	250	300	449	5
Meal 10.10–10.30												
10.10–10.50	40	..	12	18	349	41·8	63	861	103	153	216	5
10.50–11.40	50	..	12	14·4	343	41·2	49·4	108·9	131	157	206·4	5
11.40–11.55	15	..	15	60	375	56·2	224·8	108·5	164	656	880·8	5
11.55–1.30	95	..	56	35·5	483	270	171	768	430	272	443	5
1.30–3.0	90	..	47	31·5	608	285	190	702	330	220	410	4·5
3.0–3.40	40	..	18	27	626	113	170	833	149	223	393	4·5
3.40–4.10	30	..	13·5	27	640	86·5	177	903	122	244	421	4
4.10–4.40	30	..	14	28	650	91	182	953	134	268	450	4
CASE 3.												
11.30–7.30 P.M.—Meal	480	..	160	20	800	283	160	112	1792	224	384	5
7.30–7.45												
7.30–10.5	155	..	65	25·2	160	104	40·3	520	338	131	171·3	5·5
10.5–10.35	30	..	10	20	140	14	28	640	64	128	156	6
10.35–11.5	30	..	10	20	140	14	28	280	28	56	84	6·5
11.5–11.35	30	..	10	20	−8·0	−0·8	−1·6	30	3	6	4·4	6·8
11.35–12.0	25	..	10	24	150	15	36	63	63	152	188	6
Meal 12.0–12.25												
12.0–12.30	30	..	8	16	320	25·5	51	700	56	112	163	6
12.30–1.0	30	..	14	28	560	78	156	520	73	146	302	5·5
1.0–1.30	30	..	7	14	1520	106	212	400	28	56	268	5
1.30–2.0	30	..	11	22	720	79	158	860	94·5	189	347	5
2.0–2.30	30	..	12	24	800	96	192	880	156	312	504	5
2.30–3.0	30	..	11	22	1200	132	264	440	48·5	97	301	5
3.0–3.30	30	..	11	22	400	44	88	740	81·5	163	251	4·5
3.30–4.0	30	..	12	24	450	54	108	1100	122	244	352	5
4.0–4.30	30	..	9	18	480	44·2	88·4	920	82·5	165	253·4	5

TABLE I—*continued.*

	Minutes.	Alv CO_2 mm.Hg.	Volume.		Acidity (Ph 7·4) c.c. N/100 alkali.			NH_3 c.c. N/100.			A+N per hour.	pH.
			c.c. total.	c.c. per hour.	Per cent.	In whole amount.	Per hour.	Per cent.	In whole amount.	Per hour.		
CASE 4.												
9.45–6.45	600	..	175	17	515	901	90	640	1120	112	202	5
6.45–7.15	30	..	17	34	365	62	124	522	89	178	302	4·5
Meal 7.15–7.30												
7.15–7.45	30	..	12	24	262	31·9	63·8	462	55·3	110·6	174·4	5
7.45–9.30	105	..	106	61	91	96·4	55	237	251	144	199	5·5
9.30–10.0	30	..	37	74	−18	−6·3	−12·6	75	27·7	55·4	42·8	7
10.0–10.30	30	..	19	38	100	19	38	95	18	36	74	6·8
10.30–11.0	30	..	20	40	163	32·6	65·2	130	26	52	117·2	6
11.0–11.30	30	..	20	40	272	54·2	108·4	244	48·8	97·6	206	55
11.30–12.0	30	..	22	44	398	87·8	175·6	339	74·5	149	324·6	5
12.0–12.30	30	..	24	48	340	81·5	163	444	106·8	213·6	376·6	4·5
Meal 12.30–1.0												
12.30–1.0	30	..	22	44	322	70·7	141·4	324	71·2	142·4	283·8	4·5
1.0–1.30	30	..	29	58	431	125	250	564	163·5	327	577	4·5
1.30–2.0	30	..	7	14	398	27·9	55·8	237	16·6	33·2	89	4·5
2.0–2.30	30	..	11	22	547	60	120	620	68·1	136·2	256·2	4·5
2.30–3.0	30	..	13	26	585	76	152	644	83·5	167	319	4·5
3.0–3.30	30	..	7	14	870	61	122	300	21	42	164	4·5
3.30–4.0	30	..	19	38	681	129·5	259	283	53·5	107	366	4·5
Meal 4.0–4.30												
4.0–4.30	30	..	20	40	597	119·4	238·8	104·2	20·8	41·6	280·4	4·5
CASE 5.												
8.20–11.45	205	42·29	145	42·4	123	178	52·1	315	457	133	185·1	..
Meal 12.15–12.30												
11.45–1.0	75	45·58	52	416	−210	109	87	98	51	41	128	..
1.0–1.30	30	43·05	42	84	232	97	194	201	84	168	362	..
1.30–2.0	30	40·01	23	46	352	81	162	319	73	146	308	..
2.0–2.30	30	36·96	14	28	540	76	142	517	72·4	145	287	..
2.30–3.0	30	43·43	15	30	644	966	193	863	129	258	451	..
3.15	..	42·29	..	..	..	..	..	..	..	..	..	..
CASE 6.— Starvation Control.												
10.5–8.5	600	..	200	20	127	254	25·4	191	382	38·2	63·6	..
8.5–9.5	60	..	60	60	380	228	228	890	534	534	762	..
9.5–10.5	60	..	130	130	500	650	650	460	598	598	1248	..
10.5–11.5	60	..	80	80	500	400	400	430	344	344	744	..
11.5–1.5	120	..	100	50	233	233	116·5	330	330	165	281	..
Case 6.—Second Exp., Meal taken between first and second specimens.												
6.45–9.45	180	..	180	60	544	979	326·4	1044	1879	626·4	952·8	..
Meal 9.45–10.0												
9.45–11.15	90	..	120	80	104	124·8	82·2	344	412·8	275·2	358·4	..
11.15–12.15	60	..	180	180	52	93·6	93·6	144	259·2	259·2	342·8	..
12.15–1.15	60	..	55	55	37	20·35	20·35	20·4	11·2	1·2	31·5	..

In all these experiments there was either a very slight decrease or none at all in the A + N value. According to the results obtained from the all-day experiments, this would be ascribed to the latter part or acid tide of the breakfast effect.

(2) *The lunch or mid-day meal effect.*—To be sure that this meal alone is influencing the urine the subject must be starved until lunch time. Owing to the difficulty in inducing the breakfastless subject to starve until the usual lunch hour this meal was taken a little earlier than usual. Case 2 in the table is one of a series of three such experiments. The experiment commenced at 7.40 a.m.

Half-hourly specimens were collected, and the meal was taken between 12 and 1 o'clock. It will be seen that during the period of starvation the acid and NH_3 per hour steadily increase, but following the meal they begin to decrease after the first specimen, thus showing an alkaline tide. A starvation experiment was performed, the results being seen in the table, Case 6. The subject starved from 10.5 p.m. to 1.5 p.m. on the next day, hourly samples being collected after 8.5 a.m. The acidity and NH_3 per hour increase steadily for the first three hours, and then decrease, in striking contrast to the next experiment, performed on the same subject on a different day. The times were roughly the same; only a meal was taken between the first and second samples. The acidity and ammonia per hour at once fall after the meal, and continue to do so to the end of the experiment. The pH of the urine shows a remarkable rise after a meal, followed by a fall. As has been noted by many observers, it bears little or no relation to the titrable acidity of the urine.

From what has been described it can be seen that the cases examined show the alkaline tide, as first described by Bence Jones. This tide is only apparent or constant as a result of the first meal of the day. Following the alkaline tide is an acid tide, which continues from three to four hours, thus occurring in the usual lunch time. If a meal is taken during this period, the alkaline tide which would normally follow it is overshadowed, and shows itself usually by a very slight decrease in acidity. The proof that these changes are associated with meals depends on the fact that they are abolished by starvation. The alkaline tide finds a ready explanation in the secretion of gastric juice, and the acid tide in the secretion of alkaline pancreatic juice, yet there is no absolute proof of this.

Observations from a gastrectomised man were so irregular as to be worthless, and are not reproduced.

The sequence of changes fits in very well with the traditional explanation, and, since a diurnal origin is excluded by the starvation experiments, this is probably the true explanation. It was hoped that by examining men who secreted varying quantities of HCl, quantitative urinary changes would be found. Unfortunately this was not found to be the case. When the multitude of factors acting upon the urinary constituents is considered, it is not to be wondered at that the curves are rough and the changes uneven. The general metabolic processes tend in themselves to vary the reaction. Again, there is the secretion of a large quantity of gastric juice, which will tend to concentrate the urine and thus throw out calculations. That the volume of urine decreases immediately after a meal is easily seen from the tables. The juice is absorbed again in the intestines together with imbibed fluid, and again the calculations are upset. Yet through all this a definite alkaline tide appears to follow the first meal of the day.

SUMMARY.

(1) By examining specimens of urine before and after meals, it was found that the first meal of the day was followed by a decrease in the excretion of acid and ammonia calculated per hour. This alkaline tide lasted one to two hours, and was followed by a period of increased excretion of acid and alkali per hour. This acid tide lasted for about two hours, the period of the whole meal effect taking roughly from four to five hours.

(2) The changes following lunch were obscured, in the majority of cases, by the acid tide of the breakfast.

(3) Diurnal origin of these variations was excluded by starvation experiments.

(4) In view of these results, the alkaline tide was associated with gastric secretion and the acid tide with pancreatic secretion.

(5) The pH of the urine was found to rise after a meal, and to fall later.

This investigation was commenced in collaboration with Dr. E. L. Kennaway, who performed some of the urinary analyses, which I publish with his permission. I have to thank him for his advice and help throughout the investigation.

REFERENCES.

BENCE-JONES.—(1898) Quoted in Schaefer's 'Text-book of Physiology,' p. 579.
BENNETT, T. I., AND RYLE, J. A.—(1921) *Guy's Hosp. Rep.*, **71**, 268.
CAMPBELL, J. A.—(1920) *Biochem. J.*, **45**, 5.
FOLIN, O.—(1919) 'Manual of Biological Chemistry,' p. 97.
LEATHES, J. B.—(1919) *Brit. Med. J.*, **2**, 165.
MALFATTI, H.—(1908) *Zeitschr. f. anal. Chem.*, **47**, 273.
QUINCKE.—(1884) *Zeitschr. f. klin. Med.*, **7**, Suppl. 22.

TESTING THE ANTIGENIC VALUE OF DIPHTHERIA TOXIN-ANTITOXIN MIXTURES.

A. T. GLENNY, K. ALLEN, AND BARBARA E. HOPKINS.

From the Wellcome Physiological Research Laboratories, Beckenham, Kent.

Received for publication January 5th, 1923.

TOXIN-ANTITOXIN mixtures are now being widely used for the production of immunity against diphtheria. Park (1922) in New York alone has immunised 34,000 children, and in England also the practice has been adopted, O'Brien, Eagleton and Okell (1923) having already immunised some hundreds. In view of this extensive use it is obviously important that something should be known of the antigenic value of the mixtures employed.

Although Park and Zingher and others have studied the immunising efficiency of different mixtures upon groups of children, no practical laboratory method of standardisation has been available, and so far these mixtures have been standardised only according to their toxicity. The original requirements of the U.S.A. authorities were that a mixture should contain 3 L+ doses of toxin per c.c., so neutralised that 1 c.c. injected subcutaneously was harmless to guinea-pigs, while guinea-pigs injected with 5 c.c. should survive at least 10 days, but ultimately die of paralysis. These three requirements may be taken as (*a*) a standard of concentration, (*b*) a test to ensure that the mixture is not toxic in reasonable doses, and (*c*) that the toxin has not been so over neutralised as to destroy all antigenic value. It may be stated here that more toxic measures are now used in America. Park (1922) states: "Dr. Banzhaf now standardises our preparation so that 1 c.c. causes paralysis in the majority of guinea-pigs weighing 250 grm.; in all 5 c.c. causes paralysis which ends in death."

It is probable that, owing to the variations in the constitution of different toxins, the antigenic value of a mixture cannot, with any certainty, be judged by its toxicity. Efforts have therefore been made to standardise the actual antigenic value of mixtures by the rate and extent of antibody production following their injection. Four such methods of standardisation are described below.

METHOD A.

The most obvious method of testing the antigenic value of a toxin-antitoxin mixture would be to inject the mixture into experimental animals, and then to test how soon such animals were able to tolerate living culture or toxin. Tests for tolerance have the disadvantage that many test animals are needed to give quantitative results. A convenient modification of this method is to inject rabbits with the mixture under test, to withdraw blood at stated intervals, and to titrate the serum against toxin; the immunity produced by the injections of the toxin-antitoxin mixture is judged by the antitoxin produced rather than by the tolerance gained. Table I records the "primary stimulus response" of several rabbits to a single injection of four different toxin-antitoxin mixtures. Each normal rabbit was injected subcutaneously with 5·0 c.c. of the mixture under test; a few c.c. of blood were withdrawn each week or twice a week, and the antitoxic value of the blood titrated by the intracutaneous method. Rabbit G 21 eventually produced over one-half of a unit of antitoxin per c.c. of serum as the result of the subcutaneous injection of a particular mixture; two weeks elapsed before any antitoxin was detectable, and the antitoxic value gradually increased over a period of eight weeks. That the particular mixture used in this test was a good antigen was capable of easy and moderately rapid proof. The mixtures tested on the other rabbits did not produce such rapid and such marked results. Rabbits G 18 and G 23 had produced only small traces of antitoxin, difficult to detect, by the fourth week after injection, and the highest value reached was approximately $\frac{1}{30}$ and $\frac{1}{100}$ unit respectively. The fourth mixture tested failed to produce in G 20 any detectable antitoxin; this alone is not sufficient to condemn the mixture as having no antigenic value, because the existence has already been demonstrated

of active immunity to diphtheria in the absence of detectable antitoxin (Glenny and Allen, 1922[1]). Rabbit G 20 continued to show no antitoxin until at the end of 14 weeks it was injected intracutaneously with a Schick dose of dipntheria toxin. Four days later the serum of this rabbit contained $\frac{1}{100}$ unit of antitoxin per c.c.; by the ninth day the antitoxic value had reached 0·18

TABLE I.—*Showing the Antitoxic Content of the Blood of Five Rabbits after the Injection of a Toxin-Antitoxin Mixture as a Primary Stimulus.*

Rabbit	G 21	G 18	G 23	G 20	G 46
No. of mixture	1	2	3	4	5
	Units of antitoxin per c.c.				
Before injection	*nil*	*nil*	*nil*	*nil*	*nil*
1 week after	,,	,,	,,	,,	,,
1½ weeks after	—	—	—	,,	—
2 ,, ,,	0·02	*nil*	*nil*	,,	*nil*
2½ ,, ,,	0·18	—	—	,,	—
3 ,, ,,	0·22	*nil*	0·001	,,	0·001
3½ ,, ,,	0·22	—	—	—	—
4 ,, ,,	0·22	0·004	0·0015	*nil*	0·002 [A]
5 ,, ,,	0·25	0·02	0·0015	,,	0·15
6 ,, ,,	0·45	0·03	0·011	,,	0·07
7 ,, ,,	0·45	0·03	0·011	,,	0·045
8 ,, ,,	0·55	0·035	—	,,	—

Nil indicates that no antitoxin could be detected, *i. e.* less than 0·0005 unit per c.c. was present.

[A] = injection of a Schick dose of toxin.

unit. The use of the Schick test as a "secondary stimulus" (Glenny and Allen, 1922)[2] renders active immunity more easy to detect. Rabbit G 46 in Table I affords an illustration of this method of magnifying the result of the primary stimulus. Four weeks after the injection of a toxin-antitoxin mixture the serum of rabbit G 46 contained $\frac{1}{100}$ unit per c.c.; many careful titrations are needed to confirm the existence of such small amounts of antitoxin. A Schick dose of toxin was injected, and within a week the antitoxic content had reached 0·15 unit.

METHOD B.

Table II gives further illustrations of this method of magnifying the primary stimulus response. Seven rabbits were each injected subcutaneously with 1 c.c. of the same toxin-antitoxin mixture. At the end of four, eight and twelve weeks pairs were reinjected, one with a Schick dose of toxin and the other with a second dose of 1 c.c. of the same toxin-antitoxin mixture (T.A.M.). In each rabbit a marked increase in antitoxic value occurred within a week of the second injection, indicating that each rabbit was actively immune as the

result of the antigenic action of the toxin-antitoxin mixture. It is clear from Table II that the primary stimulus response to the same dose of the same mixture may vary in different rabbits. At the end of eight weeks G 35 and G 39 both showed traces of antitoxin, while G 34, G 36 and G 38 showed none; at the end of twelve weeks rabbit G 36 showed no antitoxin, G 38 showed $\frac{1}{100}$ unit, and G 39 showed $\frac{1}{60}$ unit as a result of the primary stimulus alone. The

TABLE II.—*Showing the Antitoxic Content of the Blood of Seven Rabbits after the injection of a Primary Stimulus consisting of a Toxin-Antitoxin Mixture, and a Secondary Stimulus (one, two and three months later), consisting of a Schick dose of Diphtheria Toxin or of another Toxin-Antitoxin Mixture.*

Rabbit	G 32	G 33	G 34	G 35	G 36	G 38	G 39
Primary stimulus	1·0 B. 346	1·0 B. 346	1·0 B. 346	1·0 B. 346	1·0 B. 346	1·0 B. 346	1·0 B. 346
Number of weeks elapsing between primary and secondary stimulus	4 weeks	4 weeks	8 weeks	8 weeks	12 weeks	12 weeks	—
Secondary stimulus	Schick	T.A.M.	Schick	T.A.M.	Schick	T.A.M.	—
	Units of antitoxin per c.c.						
3 weeks after primary stimulus	*nil*	*nil*	*nil*	*nil*	*nil*	*nil*	*nil*
4 weeks after	„	„	„	„	„	„	„
	injected	injected					
5 „ „	0·08	0·1	„	„	„	„	„
6 „ „	0·12	0·05	„	„	„	„	„
7 „ „	—	—	„	0·002	„	„	0·002
8 „ „	0·05	0·01	„	0·006	„	„	0·002
			injected	injected			
9 „ „	—	—	1·0	0·12	„	„	0·005
10 „ „	—	—	1·75	0·12	„	„	0·005
11 „ „	—	—	—	—	—	0·001	0·005
12 „ „	—	—	—	0·04	*nil*	0·002	0·016
					injected	injected	
13 „ „	—	—	—	—	0·06	0·05	0·02
14 „ „	—	—	—	—	·1	0·03	0·02

Nil indicates that no antitoxin could be detected, *i. e.* less than 0·0005 unit per c.c. was present.

table also shows that in each of the three pairs of rabbits tested a single Schick dose of toxin gave a better secondary stimulus response than 1 c.c. of a toxin-antitoxin mixture. The table also shows how it is simple to demonstrate in the same animal the antigenic properties of a mixture, both as a primary and as a secondary stimulus. It is of interest to record that G 34 responded to an injection of a Schick dose of toxin by the production of at least 100,000 times as much antitoxin as would be necessary completely to neutralise the toxin injected.

METHOD C.

One disadvantage of testing antigenic values by means of the primary stimulus response is the length of time elapsing before such a response occurs.

This disadvantage is lessened by using the modification recorded above in Table II, but more time can be gained by testing mixtures for the secondary stimulus response only in rabbits already immune. An increase in antitoxin content is demonstrable in four days, and high unit values are reached in from six to ten days. The advantages of this method are the rapidity with which a result can be obtained, and the great increase in the ease and accuracy of testing gained through the production of higher unit values. A marked disadvantage of the method is the introduction of additional variable factors.

TABLE III.—*Showing the Antitoxic Values of Three different Immune Rabbits after Secondary Stimuli consisting of a number of injections of the same Toxin-Antitoxin Mixture.*

Rabbit	G 8				G 11			G 17		
Number of test	1	2	3	4	1	2	3	1	2	3
Weeks since last injection of same antigen	—	15	6	8	—	6	8	—	6	8
Before injection	0·01	0·0045	0·025	0·01	0·009	0·16	0·14	0·07	0·16	0·12
1 day after	—	—	0·018	—	—	0·14	—	—	0·14	—
2 days after	—	0·0045	0·018	0·008	0·006	0·14	0·14	0·06	0·12	0·11
3 „ „	—	—	0·04	—	—	0·14	—	—	0·12	—
4 „ „	0·5	0·08	0·14	0·18	0·22	0·3	0·45	0·4	0·22	0·33
5 „ „	—	—	—	—	—	—	—	—	—	—
6 „ „	—	0·55	0·7	0·55	1·0	0·7	0·7	1·0	0·6	0·55
7 „ „	2·0	—	—	—	—	—	—	—	—	—
8 „ „	—	0·4	0·5	0·35	0·9	0·7	0·8	0·9	0·6	0·55
9 „ „	—	—	—	—	—	—	—	—	—	—
10 „ „	—	0·25	0·35	—	0·7	0·7	0·7	0·6	0·55	0·55
11 „ „	1·5	—	—	—	—	—	—	—	—	—
12 „ „	—	—	—	—	—	—	—	—	—	—
13 „ „	—	0·22	0·3	0·22	0·55	0·5	0·45	0·55	0·55	—
14 „ „	1·0	—	—	—	—	—	—	—	—	—

The extent of a secondary stimulus response depends not only upon the individual capacity of the animal, but also upon the potential activity or capability of increased response induced by former stimuli as well as upon the degree and stage of immunity of the test animal at the time of injection. It is obvious therefore that test animals must be carefully chosen if it is necessary to detect quantitative differences between the antigenic values of two mixtures. Table III records a number of tests upon the secondary stimulus response, induced by the same antigen in three different rabbits. The first test upon rabbit G 8 showed an antitoxic content of two units per c.c.; later tests of the same antigen upon the same rabbit and upon two other rabbits all showed an antitoxic content of between 0·55 and 1·0 units per c.c. The interval of time elapsing between the 1st and 2nd test on G 8 was 15 weeks; between

the 2nd and 3rd on G 8 and the 1st and 2nd on G 11 and G 17 was 6 weeks, and that between the 3rd and 4th on G 8 and the 2nd and 3rd on G 11 and G 17 was 8 weeks. The three rabbits recorded in the table exhibit considerable uniformity of response; control of certain variable factors would increase the degree of the uniformity. Thus the last two tests on both G 11 and G 17 were made when the initial values of the rabbits were between 0·12 and 0·16 unit per c.c., and within 6 to 8 weeks of the previous injection; the resultant values were between 0·55 and 0·7 unit on the 6th day, between 0·55 and 0·8 unit on the 8th day, and between 0·55 and 0·7 unit on the 10th day. The readings of the individual rabbits varied even less than this. Rabbit G 8 affords an illustration of individual peculiarity, for the 8th day reading in each case was decidedly less than the 6th day, and the rate of loss after each test was far greater than that in either of the other two rabbits. Five weeks after reaching a value of 0·55 rabbit G 8 had fallen to 0·025 compared with 0·16 unit in both G 11 and G 17 at the same interval of time after a value of 1 unit had been reached in each rabbit. Again, seven weeks after reaching a value of 0·7 unit G 8 had fallen to 0·01 compared with 0·14 and 0·12 in G 11 and G 17 after reaching 0·7 and 0·6 unit respectively. Although Table III affords good evidence that uniformity of response can be achieved, yet great variation may be obtained if conditions are not uniform, for the same dose of the same antigen in another experiment has produced as low a value as 0·12 unit in one rabbit and as high as 6·2 unit in another.

METHOD D.

The methods so far described involve injecting rabbits, bleeding and titrating certain samples of blood against diphtheria toxin, making use of guinea-pigs as indicators. These methods need special laboratory technique and the use of a number of test animals. In the method now to be described the technique is of the simplest and the number of test animals is reduced to a minimum.

For each mixture of toxin and antitoxin prepared for immunisation against diphtheria, it is necessary to determine that a definite dose is non-lethal to guinea-pigs; the size of the dose depends upon the standard of toxicity adopted. According to the earlier American requirements, guinea-pigs should survive 1 c.c. of the mixture. The simplified method of testing the antigenic value of toxin-antitoxin mixtures consists in ascertaining the degree of immunity produced in the surviving guinea-pigs which have been used to demonstrate that the mixture is harmless in certain doses. The simplest method of testing for a certain degree of immunity is to make use of the Schick reaction. In all methods of testing antigenic values it is advisable to limit the primary stimulus to a single injection, consequently the resulting immunity is small, and may not be sufficient to give a negative Schick reaction. If, however, a sufficient degree of active immunity has ensued, each injection of toxin for the Schick test will act as a secondary stimulus (Glenny and Allen, 1922)[2] and raise slightly the degree of immunity, until at some subsequent test the animal shows a negative Schick reaction. Table IV records the results of injecting a number of guinea-pigs with varying doses of a toxin-antitoxin

TABLE IV.—*Showing the Reactions produced upon Guinea-pigs by Schick Doses of Diphtheria Toxin given at varying intervals of time after the Injection of Primary Stimuli consisting of varying amounts of a Toxin-Antitoxin Mixture.*

Guinea-pig	B.	C.	D.	A.	Z.	Y.	S.	O.	N.	R.	P.	W.	X.	K.	H.	F.
Volume of T.A.M. as primary stimulus	0·2	0·2	0·2	0·2	1·0	1·0	1·0	1·0	1·0	1·0	1·0	1·0	1·0	5·0	5·0	5·0
Reaction produced by Injection of Schick Dose of Toxin.																
1 week after primary stimulus	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
2 weeks „ „	+	...	...	...	+	...	...	...	...	...	...	...	...	+	...	...
3 „ „ „	+	+	...	...	+	+	+	...	...	...	...	...	...	+	+	.
4 „ „ „	+	+	+	...	−	+	±	+	+	...	...	...	...	+	Died	+
5 „ „ „	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	Died
6 „ „ „	−	+	+	+	...	−	−	−	−	+	±	...	...	+	...	...
7 „ „ „	...	+	+	+	...	...	...	...	...	...	±	−	...	+	...	...
8 „ „ „	...	−	±	±	...	...	...	.	...	?	−	...	+	+	...	...
9 „ „ „	...	...	−	−	...	...	..	..	...	−	...	...	±	+		
10 „ „ „	...	...	...	...	...	...	...	...	...	...	..	...	−	+	...	...

TABLE V.—*Showing Tests for Toxicity and Antigenic Value of Three Different Toxin-Antitoxin Mixtures.*

Toxin-antitoxin mixture	A.						B.						C.				
Dose in c.c.	1·0	1·0	1·0	5·0	5·0	5·0	1·0	1·0	1·0	5·0	5·0	5·0	1·0	1·0	5·0	5·0	5·0
Reactions produced by Schick Dose of Toxin.																	
1 week after primary stimulus	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
2 weeks „ „	...	...	...	Died 11th day	...	...	...	...	...	...	...	...	...	...	...	...	...
3 „ „ „	+	+	+	...	Died 22nd day	Died 24th day	+	+	+	Died 24th day	Died 24th day	Died 26th day	+	+	+	+	+
4 „ „ „	−	−	−	...	...	...	+	+	+	...	...	...	+	+	+	+	+
5 „ „ „	...	...	...	...	...	...	+	+	+	...	...	...	−	+	+	+	+
6 „ „ „	...	...	...	...	...	...	+	+	+	...	...	...	...	±	+	−	+
7 „ „ „	...	...	...	...	...	...	−	−	+	...	...	...	...	−	−	...	+
8 „ „ „	...	...	...	...	...	...	...	...	−	...	...	...	...	...	...	...	+

mixture and then testing them with Schick toxin each week, commencing two, three, four or six weeks after the injection of the mixture. Of four guinea-pigs injected with 0·2 c.c. of T.A.M. all gave at least three positive reactions before their immunity was sufficiently raised to give a negative reaction. Of the nine guinea-pigs injected with 1·0 c.c. of T.A.M. none needed more than two injections of Schick toxin before the reaction became negative. In both groups the degree of immunity represented by a negative Schick reaction was attained at a stage determined by the number of previous injections of Schick toxin, rather than by the time interval after the primary stimulus. We have adopted as a measure of immunity conferred, and consequently of antigenic values of mixtures injected, the number of weekly Schick tests required before a negative reaction occurs, the injections of Schick toxin being commenced three weeks after the primary stimulus. It should be noted that two of the three guinea-pigs injected with 5·0 c.c. of the mixture died of diphtheritic paralysis four or five weeks after injection. The surviving guinea-pig showed signs of paralysis. This guinea-pig failed to respond to injections of Schick toxin and was less immune than those that had received only $\frac{1}{5}$ c.c. of the same mixture. It has been found frequently that excessive doses of T.A.M. producing poor condition fail to produce immunity, and further that in a group of guinea-pigs injected with the same dose of the same antigen, one in poor condition from any cause may respond far less readily than others in the same group.

Table V shows comparative tests for toxicity and antigenic value of three different toxin-antitoxin mixtures. Three guinea-pigs were injected with 1·0 c.c. and three with 5·0 c.c. of each mixture. Mixtures A and B were of similar toxicity; guinea-pigs injected with 5 c.c. of A died in 11, 22 and 24 days, those injected with 5 c.c. of B died in 24, 24 and 26 days. Marked differences were seen in their antigenic values; the three guinea-pigs injected with 1·0 c.c. of A gave negative reactions at their 2nd Schick injection; those that had received 1·0 c.c. of B gave negative reactions at the 5th, 5th and 6th injection. Again, when mixtures B and C are compared, discrepancies are seen between toxicity and antigenic value; C was less toxic than B in that all three guinea-pigs injected with 5·0 c.c. of C survived, yet the two animals receiving 1 c.c. of C showed higher immunity, yielding negative reaction at the 3rd and 5th (reduced at 4th) injection compared with 5th, 5th and 6th with 1 c.c. of B. The guinea-pigs injected with 5 c.c. of C although surviving without signs of paralysis were sufficiently affected in general condition to fail to respond more readily than those injected with 1 c.c.

SUMMARY.

Evidence is brought forward to show that the toxicity test alone may give incomplete or erroneous conclusions as to the immunising value of a toxin-antitoxin mixture intended for immunisation against diphtheria.

Four methods are described for testing the antigenic value of mixtures:

(*a*) Tracing the antitoxic value of the blood of rabbits after a single subcutaneous injection of the mixture as a "primary stimulus."

(*b*) Accelerating the results of (*a*) by a subsequent injection, as a

"secondary stimulus," of either the same mixture or of a Schick dose of toxin.

(*c*) Tracing the increase in antitoxic values of the blood of immune rabbits after a subcutaneous injection of the mixture as a secondary stimulus.

(*d*) Injecting guinea-pigs, which have survived the test for toxicity of the mixture, with Schick doses of diphtheria toxin at weekly intervals commencing three weeks after the injection of the mixtures.

REFERENCES.

GLENNY, A. T., AND ALLEN, K.—(1922)[1] *J. Hyg.*, **21**, 100.—(1922)[2] *Ibid.*, **21**, 104.
O'BRIEN, R. A., EAGLETON, A. J., OKELL, C. C., AND BAXTER, M.—(1923) *Brit. J. Exper. Pathol.*, **4**, 29.
PARK, W. H.—(1922) *J. Amer. Med. Assoc.*, **79**, 1584.

THE "GASTRIN" CONTENT OF THE HUMAN PYLORIC MUCOUS MEMBRANE.

S. E. AMMON AND R. K. S. LIM.

From the Department of Physiology, Edinburgh University.

Received for publication January 9th, 1923.

ASSUMING that the human pyloric mucosa yields a gastric excitant or gastrin on acid extraction, similarly to other mammals, we intended to examine the gastrin content of the mucous membrane in pathological conditions and compare it with the normal. We use the term "gastrin," not in the sense in which it was coined by Edkins (1905, 1906), viz. to denote a gastric hormone, but merely as an excitant obtainable from the stomach and capable of stimulating gastric secretion on intra-vascular injection.

It was unfortunate, but unavoidable, that the material could only be obtained from the post-mortem room many hours after death.

The pyloric mucous membrane was dissected off, and on most occasions prepared by boiling with HCl, etc., as for the extraction of secretin. In a few cases the method of Dale and Laidlaw (1912) was utilised.

We employed the method devised by one of us (1922)[1] for the observations on the gastric secretion, the experimental animal being the cat.

The results are shown in Table I.

TABLE I.

	Condition of stomach.	Condition of individual. Age.	Sex.	Disease.	No. of hours between death and preparation of extract.	Method of extraction.	Effect on gastric secretion.
1	Pylorus normal	?	F.	Carcinoma (cervix uteri)	48 hours	HCl	No secretion.
2	Pylorus normal: recent ulcers in fundus	70	M.	Lymphosarcoma (mediastinum)	48 „	„	Marked secretion.
3	Pylorus normal	62	F.	Carcinoma (pelvic colon)	14 „	„	No secretion.
4	Pylorus congested	60	F.	Endocarditis	59 „	„	„
5	Pylorus normal	35	M.	$CHCl_3$; cardiac failure	50 „	„	Slight secretion.
6	„ „	41	F.	Mastoid abscess pyæmia	12 „	„	No secretion.
7	„ „	56	M.	Chronic interstitial nephritis	28 „	D. & L.	„
8	„ „	34	M.	Meningitis	24 „		
9	„ „	18	F.	Acute lupus erythematosus	63 „	D. & L.	Secretion?
10	„ „	64	M.	Myocarditis and nephritis	36 „		

HCl, extraction by boiling with 0·4 per cent. HCl, etc.; D. & L., extraction by the method of Dale and Laidlaw (1912).

From Table I it will be seen that with one exception (Case 2, Fig. 1) all our extracts showed little or no activity. They were not nearly so active as similar preparations from smaller animals, viz. the cat and dog. The exception mentioned was prepared from a stomach which showed signs of recent acute ulcers. This was the only stomach showing ante-mortem local changes.

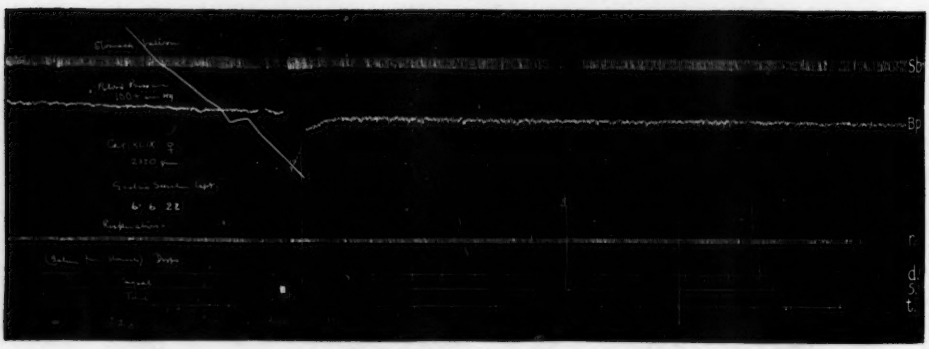


FIG. 1.—Tracing showing the effect of injecting 2 c.c. of pyloric extract from Case 2. The injection was given just after the stomach had been washed out with saline. The drops indicated on the left of the tracing are residual drops of saline. *sb*, balloon tracing; *bp*, blood-pressure; *r*, respiration; *d*, drops of secretion from stomach; *s*, signal; *t*, time in minutes.

It would appear from the negative character of our results either that there is little gastric excitant normally present in the human pyloric mucosa, or that the excitant becomes destroyed by the changes occurring after death. Against the latter argument is the commonly accepted opinion that the mortifying intestine engenders the formation of histamine, a base which should have given us some effect on the gastric secretion (Lim, 1922[2]). Apparently no histamine was formed. At any rate, post-mortem changes had not affected the activity of the preparation made from Case 2 forty-eight hours after death. It may be that the presence of the gastric excitant is in some way related to the ulceration found in the stomach. It would seem, therefore, that the human pyloric mucosa contains little or no gastrin, and certainly much less gastrin than is found in the lower animals.

The expenses incurred have been defrayed by grants from the Earl of Moray Fund, Edinburgh University, and from the Carnegie Trust.

REFERENCES.

DALE, H. H., AND LAIDLAW, P. P.—(1912) *J. Physiol.*, **44**, xi.
EDKINS, J. S.—(1905) *Proc. R. Soc.*, B, **76**, 376.—(1906) *J. Physiol.*, **34**, 133.
LIM, R. K. S.—(1922)[1] *Quart. J. Exp. Physiol.*, **13**, 71.—(1922)[2] *ibid.*, **13**, 79.

THE SCHICK TEST AND ACTIVE IMMUNISATION.

R. A. O'BRIEN, A. J. EAGLETON, C. C. OKELL AND MISS M. BAXTER,

From the Wellcome Physiological Research Laboratories, Beckenham, Kent.

Received for publication January 10th, 1923.

IT will probably be some considerable time before the people of any large city in England are so alive to the importance of the more recent measures for the control of diphtheria that they will freely allow their children to be tested by the Schick method, and if found to be positive reactors to be given the three doses of a toxin-antitoxin mixture. In the remarkable campaign carried out recently in New York some ninety thousand children were tested and if positive immunised, 70 per cent. of the parents giving consent.

However, these new measures are slowly but surely being adopted in England, and a review of the results obtained is probably sufficiently important to merit publication at this stage. Leete (1920), Ward (1921), Dickinson (1922), Harries (1922), Dudley (1922), and Copeman with the present authors (1922) have given the results of Schick tests.

To these series we have to add tests on 2769 individuals (see Table I).

TABLE I.—*Results of Schick Tests.*

Adults.	Number tested.	Percentage negative.
Medical students—a London teaching hospital	160	35
Nurses—a London teaching hospital	48	49·5
Nurses—School A	182	78
„ „ B	59	74
Children.		
School B	739	72
„ C	329	69
An institution for children	1046	62
Good class private school	53	28
Infant welfare centre	153	24

The results closely resemble those obtained elsewhere, the groups from a "higher" social stratum showing a lower degree of immunity than those from a "lower," while the children, mostly aged from one to five years, from the infant welfare centre came from a district in which there has been but little diphtheria for some time past.

Apparently the children of the well-to-do are carefully guarded in their early lives, and escape the chance of the frequent mild attacks of unrecognised diphtheria which would otherwise eventually cause them to become immune. In a similar manner the children of intelligent artisans who live in well-kept homes in a district where few cases of diphtheria have occurred in recent years "escape" or "avoid" natural immunisation.

Age distribution.—Our general results agree with those reported in the literature, the immunity rate rising during the years of childhood and becoming constant in early adult life. The deviations in each individual group are associated with the special conditions of the group, or in some cases are probably due to the smallness of the statistical units.

Familial immunity.—As observations accumulate it may become possible to decide whether there is a family factor in the readiness with which immunity is developed. Unfortunately the observations hitherto recorded are insufficient to throw much light upon this point. When immunising 107 families, we found in 20 that the children were positive reactors; in 54 all negative; in 20 the older negative and the younger positive in accordance with Zingher's observations; and in 13 (12 per cent.) the older children positive, while the younger were negative. The members of these 13 families did not live together during the whole of their lives.

In one group of 136 children, all of whom originally gave a Schick-positive reaction, there were only three individuals who failed to give a negative Schick reaction within 11 weeks of the commencement of the "immunisation"; of these two were brother and sister, and both proved refractory until 21 weeks had elapsed. The remaining one was still Schick-positive after this interval.

Twins.—We tested three pairs of twins; each twin gave the same reaction as the other in two cases; the twins were similar and less than three years

old. Of a third pair of dissimilar twins, aged 7 years, the boy was a positive reactor and the girl negative.

RESULTS OF IMMUNISATION.

The routine procedure was to give a weekly intramuscular injection of 1 c.c. of toxin-antitoxin mixture until three doses had been given. At different intervals after the last injection small groups of these children, as they became available, were subjected to the Schick test.

TABLE II.—*Results of Immunisation.*

	Weeks elapsed between last injection of toxin-antitoxin mixture and first negative response to Schick test.							
	1	2	3	4	5	6	7	8
School B:								
Initial immunity rate 72 per cent.								
Number tested .	19	56	37	4	20	3	1	1
,, negative .	17	46	31	4	16	3	1	1
Percentage negative	89	82	84	100	80	100	100	100
School C:								
Initial immunity rate 69 per cent.								
Number tested .	—	—	—	13	38	30	15	5
,, negative .	—	—	—	9	32	24	15	5
Percentage negative	—	—	—	70	84	80	100	100
Institution for children:								
Initial immunity rate 62 per cent.								
Number tested .	12	24	30	53	9	133	2	—
,, negative .	6	14	24	29	8	87	2	—
Percentage negative	50	58	80	54	89	65	100	—

The groups are very small and the statistical error therefore great.
A summary of these results is given in Table III.

TABLE III.

Place.	Initial immunity percentage.	No. of positive reactors immunised and re-tested.	No. giving negative Schick test.	Weeks since last injection of mixture.	Percentage Schick-negative.
Institution for children	62	348	296	11	83
School B	72	136	133	11	98
School C	69	101	92	8	91
	—	—	101	20	100

It may be of value to present the evidence in another way.
A group of 136 "positive reactors" was injected and re-tested. Within

8 weeks 129, *i.e.* 94 per cent., showed a negative Schick reaction; thereafter the results were as follows, the figures in brackets giving the number yielding a Schick-negative reaction at the expiry of the corresponding number of weeks: 9 (2), 10 (1), 11 (1), 21 (2). One child, though reinjected, remained positive; we hope to be able to re-examine him later.

With regard to the permanence of the negative reaction in immunised children, we had the opportunity of re-testing a group of 203 children, both those originally negative to the Schick test, and also those who had become negative after immunisation. When re-tested 11 weeks later the reactions agreed in 201 cases; 2 who had been negative now gave a positive reaction. Inquiry revealed that these children had had antitoxin injected some few weeks before the first test was carried out.

To one question of interest we have only a partial answer. From our general knowledge of immunity it is to be expected that a school population showing a high percentage of Schick-negative reactors and having a recent history of diphtheria would be one easily immunised. This anticipation is realised in the large groups of figures given by Zingher. We know also from Dudley's careful work (1922) that a population of boys exposed to an epidemic of diphtheria will show a high percentage of "Schick-negative reactors." It would be of great scientific interest to know how many of the children would have become immune without the injection. Probably some few of them would. We have had unfortunately, in this, as in so many other aspects of this work, very restricted opportunities of making observations under satisfactory conditions; however, at one school, 19 children who gave Schick-positive reactions did not become available to us again for seven months, during which time the remainder of the positive reactors in the school were immunised. All of these 19 children were now injected. Five weeks after the completion of the course of injection these children were again tested; 5 of them gave a positive response. Thus, this group of 19 children who had been in contact with the other children and had also recently had prophylactic injections showed an immunity rate of only 74 per cent., whereas the children who had been fully immunised at this time gave a 99 per cent. immunity rate. Though the figures are small, they indicate that the increased immunity rate in the whole school was almost entirely the result of the toxin-antitoxin injections.

The figures from the welfare centre may be of further help. Of these children only 24 per cent. were Schick-negative reactors when they were first examined. Of 33 children tested, found positive and injected with mixtures, 31, *i.e.* 93 per cent. became Schick-negative reactors within 12 weeks. During this period no cases of diphtheria were notified within the area. Small groups of fresh children were still being tested each week, and the immunity rate was still between 25 and 30 per cent.

It is reasonable to conclude that in this particular population, the greater part of the conversion of the "Schick-positive" population to a "Schick-negative" one was due to the injections given.

Reactions after injection of toxin-antitoxin mixture.—The reactions in these groups of children have been very slight: with the exception of three instances in our early work we have no record that any child developed a

reaction and illness sufficiently severe to cause it to be confined to bed for one day. The great majority suffered nothing but a temporary slight soreness of the arm, and very few had any general reaction.

SUMMARY.

(1) The results of somewhat over 2700 Schick tests are given. The percentage immunity at various ages agrees generally with observations in the literature. The "higher" the social status of an urban population group, the lower will its immunity rate be.

(2) The results of the immunisation of 585 positive reactors are given. Between 85 per cent. and 98 per cent. of the positive reactors in the different groups became negative to the Schick test in less than three months.

(3) No serious reactions after injections of the toxin-antitoxin mixtures were observed amongst these children.

REFERENCES.

COPEMAN, S. M., O'BRIEN, R. A., EAGLETON, A. J., AND GLENNY, A. T.—(1922) "Experiences with the Schick Test and Active Immunisation against Diphtheria," *Brit. J. Exper. Pathol.*, **3**, 42.

DICKINSON, T. E.—(1922) "The Schick Test for the Determination of Susceptibility to Diphtheria: a Record of 1200 Cases," *Lancet*, **1**, 312.

DUDLEY, S. F.—(1922) "The Relation of Natural Diphtheria Antitoxin in the Blood of Man to previous Infection with Diphtheria Bacilli," *Brit. J. Exper. Pathol.*, **3**, 204.

HARRIES, E. H.—(1922) "The Carrier of Diphtheria Bacilli from the Fever Hospital Standpoint," *Med. Officer*, **27**, 91.

LEETE, H. M.—(1920) "The Schick Reaction," *Lancet*, **1**, 192.

PARK, W. H.—(1922) "Toxin-antitoxin Immunisation against Diphtheria," *J. Amer. Med. Assoc.*, **79**, 1584.

WARD, G.—(1921) "The Schick Reaction," *Brit. Med. J.*, **1**, 928.

THE DIAGNOSTIC VALUE OF RABBIT INOCULATION IN *ENCEPHALITIS LETHARGICA.*

JAMES McINTOSH.

From the Bland-Sutton Institute of Pathology, Middlesex Hospital, London.

Received for publication January 11th, 1923.

MANY cases of *encephalitis lethargica* present considerable difficulty in their diagnosis, and it was with the idea of overcoming this that the following observations were made. The amount of material at my disposal was small, but it is thought desirable that the investigations should be put on record.

The work may be regarded from two standpoints:

(1) The relative infectivity of the brain emulsions as compared with the cerebro-spinal fluid.

(2) The significance to be attached to the histological changes found in the rabbit brain.

In a former article (1918) I pointed out the general insusceptibility of the lower monkeys to the virus of *encephalitis lethargica*—a condition of affairs which is just the opposite of what occurs in poliomyelitis. Some two years ago Loewe, Hirshfeld and Strauss (1919) showed that *encephalitis lethargica* could readily be transmitted to rabbits by intracerebral inoculation, and my own observations (1920) have convinced me that the rabbit is a much more suitable animal than the monkey, hence all the experiments detailed below were performed on rabbits.

For the injections young rabbits of about 1500 grm. were used, and the inoculations were made intra-cerebrally in the following manner: A small incision was made to the left of the middle line and just behind the level of the mid-interocular line. A small hole was then made by means of a drill (1·0 mm.) and 0·25 to 0·5 c.c. of the suspected brain emulsion injected. The emulsion was prepared by emulsifying about 5 grm. of brain tissue in 20 c.c. of normal saline. Pieces of the basal nuclei removed *post mortem* were preserved in 33⅓ per cent. glycerine till required. In the case of cerebro-spinal fluid 0·5 c.c. was injected intra-cerebrally and 4 c.c. subcutaneously.

The experimental disease in rabbits shows considerable variations in its clinical manifestations; occasionally paralyses, tremors and convulsions are evident, while others die suddenly with no previous sign of ill-health. As a rule the symptoms appear about 48 hours after the injection is made. The temperature is raised 2 to 3° F., the animals are restless and excitable, choreiform movements appear; a day or two later the temperature falls and the animal becomes paretic and dies. The average duration of the infection is five or six days. In a few animals the disease is fulminating and death may occur on the second or third day; in others no symptoms appear at all and the animal is found dead several weeks later.

No lesions are found on macroscopical examination, but microscopically the brain shows changes, the true diagnostic significance of which will be discussed later. The lesions consist of a small round-cell infiltration of the meninges and of the perivascular lymph-sheaths, while small inflammatory foci in the brain substance are not uncommon.

RELATIVE INFECTIVITY OF ENCEPHALITIS MATERIAL.

The results are best given in tabulated form:

Exp. No.	Material.	Identification.	Result of inoculation.	Date.	Remarks.
1	Cerebro-spinal fluid	U.C.H.	Negative	28.12.20	
	Brain emulsion	U.C.H.	Positive	6.1.21	
	Tonsil (filtered)	U.C.H.	Positive	6.1.21	
2	Brain emulsion	M.H.	Negative	6.1.21	Infant. Brain lesion not characteristic. Case recovered.
3	Cerebro-spinal fluid	M.H.	Negative	18.1.21	
4	Cerebro-spinal fluid	M.V.	Positive	4.2.21	One rabbit died 9.9.21, extensive infiltration in brain.
	Brain emulsion	M.V.	Positive	9.3.21	
5	Brain emulsion	T.C.	Negative	29.3.21	Brain infected when received.
6	Brain emulsion	S.M.H.	Positive	29.3.21	

Note.—In every instance the cerebro-spinal fluid was inoculated into Noguchi medium without any evidence of subsequent growth.

From the above table it will be seen that three out of five gave positive results on inoculation when brain emulsions were used, while the cerebro-spinal fluid gave negative results except in one case. One animal, in Experiment No. 4, died seven months after inoculation with the cerebro-spinal fluid, and showed a doubtful infiltration of the meninges. Loewe and Strauss (1920), on the other hand, recorded 12 positive results in 16 cases, but they were working during the height of an epidemic. More recently Kling and collaborators (1921) found changes in the brains of two rabbits which had been inoculated with cerebro-spinal fluid from a case of encephalitis. No clinical symptoms were observed in the animals, but the characteristic lesions were found when the animals were killed 38 and 40 days later. Evidently the animal should be killed some time after inoculation and the brain examined, as if disease results it may be mild and recovery apt to take place.

SIGNIFICANCE OF HISTOLOGICAL LESIONS.

In the earlier part of this article it is indicated that in the diagnosis of experimental encephalitis, owing to the variability of the clinical signs, chief reliance is placed on the histological changes found in the brain. It is proposed here to discuss the true significance of these.

In the first place it has been known for a long time that the rabbit is extremely sensitive to intracerebral inoculations. Thus intracerebral inoculations of small doses of bacteria which are harmless when inoculated elsewhere will produce a rapid and fatal meningitis, while the injection of small

amounts of such a relatively harmless substance as sterilised indian ink may produce fatal results in young rabbits.

Quite recently Oliver (1922) has drawn attention to the occurrence of a spontaneous chronic meningo-encephalitis in rabbits. The lesions observed consist of an infiltration of the meninges and of the vascular lymph-sheaths with round cells, chiefly lymphocytes and a few plasma-cells. No micro-organisms were demonstrable in the brain. Oliver draws attention to the similarity of the lesion to that found in man in *encephalitis lethargica*. These infections in rabbits may be related to the spontaneous encephalitis of horses (Borna's disease), which recently has been shown to be transmissible to rabbits by experimental inoculation (Moussu, 1922).

Secondly, other filter-passing viruses tend to produce a somewhat similar histological change, viz. poliomyelitis, rabies, vaccinia, nervous herpes. Poliomyelitis, however, can only be transmitted to rabbits with the greatest difficulty, and when accomplished, many polynuclear leucocytes are to be found in the infiltrations. Rabies is an unlikely possibility, and the previous history of the case from which the material was derived should be sufficient. Vaccinia virus, as was recently shown by Levaditi and Nicolau (1921), can produce changes in the rabbit's brain almost indistinguishable from those of encephalitis, but I have found that although a primary infection in the cerebrum is obtained, it is only with the greatest difficulty that the virus can be carried on in series by brain inoculations alone. The cellular infiltration is more meningeal than perivascular and contains many large mononuclear cells; on the other hand, in *encephalitis lethargica* the cellular infiltration consists mainly of lymphocytes and glial cells.

CONCLUSIONS.

From the above observations it would appear that rabbit inoculations are of the greatest value in confirming a diagnosis of *encephalitis lethargica*.

Emulsions made of the basal nuclei give in most cases a positive result in the rabbit, while cerebrospinal fluid was only positive in one out of four cases.

It seems probable, from the findings of Kling, that a higher percentage of positive findings might be obtained with cerebrospinal fluid if the rabbits which show no signs after inoculation were killed 10 to 20 days later and the brain examined histologically.

In conclusion, it is necessary to impress on the observer that the important features of the lesion are (1) the site, (2) the nature of the cells, and (3) the presence of small foci of cells in the brain substance.

REFERENCES.

KLING, C., DAVIDE, H., AND LILJENQUIST.—(1921) *Compt. rend. Soc. de biol.*, **85**, 823.
LEVADITI, C., AND NICOLAU, S.—(1921) *Compt. rend. Acad. des sci.*, **173**, 870.
LOEWE, L., HIRSHFELD, S., AND STRAUSS, I.—(1919) *J. Infect. Dis.*, **25**, 378.
LOEWE, L., AND STRAUSS, I.—(1920) *J. Amer. Med. Assoc.*, **74**, 1373.
McINTOSH, J.—(1918) *Rep. Local Government Board on Public Health and Med. Subjects*, No. 121, London (H.M. Stationery Office), p. 57.—(1920) *Brit. J. Exper. Pathol.*, **1**, 257.
MOUSSU, R.—(1922) *Rec. méd. vét.*, **98**, 499.
OLIVER, J.—(1922) *J. Infect. Dis.*, **30**, 91.